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EFFECT OF STRESS ON NUTRIENT REQUIREMENTS OF MAN

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A B S T R A C T

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"Effect of Stress on Nutrient Requirements of Man"

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Eleven M.I.T. freshmen were given a formula diet containing 14% protein, 50% carbohydrate and 36% fat calories to supply the protein and calorie intake to which they were accustomed. Complete urine and fecal collections were obtained during ~~three~~ consecutive 3-day periods during the second semester and for ~~two~~ consecutive 3-day periods during the week of final examinations. Analysis of variance of both nitrogen balance and urinary nitrogen excretion showed no significant differences within the basal periods or the two stress periods, but did show a highly significant decrease in nitrogen balance and increase in nitrogen excretion during the stress periods. In 8 of the 11 students, nitrogen balance became negative in at least one of the stress periods. Nitrogen absorption was not affected. No correlation with urinary 17-hydroxy steroid excretion was observed. No significant effect on the absorption of calcium, phosphorus, sodium or potassium was noted. Although there was a tendency for urinary excretion of these to increase, the data were not adequate to establish the significance of this observation.

Five of these subjects were re-studied for ~~three~~ 3 additional consecutive 3-day basal periods in October. Results averaged 10.98 gm. of urinary nitrogen per day compared with 11.83 during the spring basal periods and 14.27 during the stress periods for these same subjects. Thus, urinary nitrogen excretion values during the stress of the examination period are significantly higher than basal periods either before or after.

Introduction

In the course of metabolic balance experiments with human subjects, most experienced observers have identified periods of unexpectedly poor results which they believed were associated with emotional or psychological factors in the subjects. Usually these are sporadic observations for which proof of the responsible factors is lacking and which are not reported in the literature.

An example of this type of observation is the case of the woman who had been in nitrogen equilibrium for several months on a daily intake of 1,800 calories and one gm. of protein per kilo until she learned that her son had been injured in battle in Korea. Although her food intake was unchanged and she showed no obvious agitation, her nitrogen balance became strongly negative and remained so for a week until she received further news that he was recovering. This would not have been reported except that it was elicited by direct questioning at a conference where the discussion was taped and published (M.A. Ohlson, Symposium on Protein Nutrition, Ann. N.Y. Acad. Sci. 69:913, 1958).

Persons studying calcium balance have also found an adverse effect of emotional stress on retention. This has been observed by Stearns *et al*. in children (ibid. 69:857, 1958), in some unmarried mothers (Stearns, unpublished data, 1962) and in a young graduate student whose calcium balance became increasingly negative as his preliminary doctorate examination approached and correspondingly positive after he had successfully passed it (O.J. Malm, Calcium Requirements and Adaptation in Adult Men, Oslo University Press, 1958).

Since personnel of the Armed Forces are frequently subjected to a variety of psychological stresses, the present project was initiated to determine in a systematic manner on an adequate number of subjects the quantitative effects of a number of specifically identifiable stresses of a psychological nature. These are to include examinations, sleeplessness and reversal of night and day.

Subjects who contract infections during the project which are not severe enough to require hospitalization are being studied for the parallel effect of this type of stress on nutrient balance. A companion part of the project, financed primarily by N.I.H. Grant No. A-6274 places special emphasis on this type of stress.

As applied to psychological stress even the term "identifiable" is subject to some controversy since it is well known that a given situation may be stressful for one person and not for another. While there are few who enjoy the final examinations of their freshman year at M.I.T., they are certainly more stressful for some students than for others. Psychiatric tests and interviews have been used to attempt to determine the degree of stress involved for each individual. While the results are described in one of the following sections of the report, such effects have, thus far, proved of limited value.

Time Schedule: Funds did not become available until February 1, 1962 and delivery of key basic laboratory equipment items was not completed until late May. The first group of students was selected in February. Physical and basic psychiatric tests were completed during March and baseline metabolic data obtained during April and May.

The project to date has measured only the effects of psychological stress as represented by the final examinations at the end of the academic year in randomly selected M.I.T. freshmen. The stress period for all subjects was May 26 through June 1. Biochemical analyses on stored samples were completed during the summer. Supplementary baseline data on a subsample of this initial group was obtained in October, as well as baseline data on a new and larger group of subjects.

Subjects

1. Selection: At the suggestion of the psychiatric consultant, an effort was made to obtain a random sample, rather than a volunteer group. It was thought that persons who felt themselves under stress might be less likely to enter such a program if volunteers were called for. A letter was sent to 25 freshmen drawn randomly from the list of names of the freshman class. This class was chosen because it was the first to receive extensive preliminary psychiatric testing, because its members would be available for the longest time period for continuing studies and because the final examinations of the freshman year constitute more of a stress for a large proportion of the students than those of subsequent years.

The initial letter was answered by 13 students. Of these, 12 agreed to participate in the study; the remainder had living arrangements or personal schedules that interfered with their participation. One of the 12 subsequently dropped out of the study on the second day of the baseline period because of difficulties with fecal collections and social pressure in his fraternity house. More students could not be included in the study in the time

available, but follow-up letters were sent to those not responding to the original letter to find out why they had not replied and when necessary, telephone calls were made. One boy indicated that he did not want to restrict his diet; three intended to reply but procrastinated until it was too late and eight felt too busy to undertake anything more. There was no evidence that there was any difference in concern with studies and examinations or in adjustment to the Institute among those who responded and those who did not. In other words, the non-responders did not differ in any consistent or clearly identifiable way from those who answered the initial letter.

No urine sample was found to have gross quantitites of either glucose or albumin and microscopic examination of the sediment from a centrifuged urine specimen failed to reveal evidence of any genito-urinary disorder. Baseline data are given in Table 1.

Each subject was examined by the same experienced psychiatrist soon after completion of his basal periods. In the course of an open end-type of interview lasting one to two hours, a more or less standardized series of enquiries were made. Interviews were taped and reviewed before the preparation of a final report. The gross findings of the interview are summarized in Table 2. Such a summary is, however, an over-simplification of complex and often ambiguous information. Neither in this study nor in the extensive testing of the freshman class did the psychiatrist feel able to select with certainty those students who were experiencing the greatest psychological stress.

2. Characteristics: For all of the subjects the medical history was reviewed and a careful physical examination made immediately prior to the basal period; this supplemented the medical history and physical findings recorded at the beginning of the academic year. Almost the only positive finding was mild acne of the face in 6 of the group. The age, height and weight of each subject are listed in Table 1 along with positive findings on the physical examination.

The white blood cell count and sedimentation rate measurements helped to rule out any infectious process. The hematocrit ruled out anemia and was used to correct the sedimentation rates. Initially urine specific gravity for KGJ was 1.006, but on subsequent examination it was between 1.028 and 1.026 with a pH of from 5.0 to 7.0.

Table 1.

Summary of Initial Physical and Laboratory Findings

Subject	Age	Height	Weight	White Blood Count	Sedimentation Rate mm/hr	Hematocrit %	Positive Findings in Physical Exam
W.K.F.	19	6'0"	70.9	5,900	3	48	Moderate facial acne, Fungus infection ear canals
V.R.J.	19	6'0"	89.1	10,600	1	46	Mild facial acne
S.D.C.	19	5'4"	65.5	7,000	-	42	None
P.O.Q.	19	5'9"	78.2	4,900	1	45	None
M.O.M.	19	6'1½"	76.4	5,700	1	44	Slightly enlarged posterior cervical nodes
[illegible].S.J.	19	5'10"	65.5	6,500	6	47	None
M.W.G.	19	5'10"	81.	7,900	1	46	Mild facial acne
K.M.D.	19	6'1"	69.1	8,400	2	47	Moderate facial acne
[illegible].G.J.	19	6'0"	67.3	5,200	3	47	Moderate facial acne
S.J.C.	18	5'9"	65.5	8,600	2	44	None
[illegible].O.K.	19	5'8"	60.0	6,200	8	46	Moderately severe facial acne, Borderline goiter
C.J.J.	19	5'7"	66.4	7,500	5	45	None

Table 2.

Summary of Impressions From Psychiatric Interview

Subject	Adjustment to MIT	Academic Status	Motivation	Apparent Reaction to Exams
W.K.F.	Borderline	Satisfactory	Social	Marked anxiety
V.R.J.	Unsatisfactory	Unsatisfactory	Curiosity	Considerable anxiety
S.D.C.	Satisfactory	Satisfactory	Scientific	Mild anxiety
P.O.Q.	Unsatisfactory	Borderline	Diet, Social, Financial	Considerable anxiety
M.O.M.	Very satisfactory	Very satisfactory	Financial, Social	Mild anxiety
L.S.J.	Satisfactory	Satisfactory	Financial, Social	Anxiety
M.W.G.	Very satisfactory	Very satisfactory	Financial	?Minimal anxiety
K.M.D.	Satisfactory	Satisfactory	Social	High anxiety
K.G.J.	Very satisfactory	Very satisfactory	Scientific	Mild anxiety
C.O.K.	Satisfactory	Very satisfactory	Scientific, ?Social	No "apparent" anxiety
C.J.J.	Borderline	Satisfactory	Scientific	Mild anxiety

Experimental Design

A. Procedures

The random selection of the subjects from the M.I.T. freshman class and their characteristics have been described. The purpose of the studies and importance of adherence to the diet and of complete urine and fecal collections were emphasized in recruiting and briefing the group. They were instructed to report any mistakes, lapses or difficulties of any kind to one of the two dietitians who saw them at least twice a day and who also questioned each subject as to his daily progress and reactions to the study.

The basal or control period consisted of three consecutive 3-day periods although it was intended that the first 3 days serve as a period of adjustment to the diet and of training in the collection procedures. During the examination week subjects were studied for two consecutive 3-day periods. Each 3-day period began with breakfast on the first day and ended immediately before breakfast on the fourth day.

A capsule containing a carmine marker was given at the beginning of the experiment and at the end of each 3-day period. Fecal collections were started when the dye first appeared in the feces and ended when the dye from each succeeding carmine capsule first appeared. Occasionally stool samples were unduly delayed due to small fecal volume so that the elapsed time between successive collection periods varied considerably.

Urine collections were timed by asking the subject to begin daily collections with the sample after the first one voided in the morning. Thus, at the beginning of a series the first sample of the morning was discarded and the time noted at the start of the collection period. On each succeeding day the first sample voided became part of the collection for the preceding 24 hours.

At all times subjects carried with them containers for the collection of urine and feces and specimens were refrigerated as soon as possible, usually in less than one hour. One or two blood samples for total protein and vitamin C determinations were obtained by finger tip puncture.

Subjects consumed their morning and noon meals under the direct supervision of one of the dietitians. Most evening meals were also consumed in the experimental kitchen, but subjects were allowed to take their evening meal and a mid-evening snack home with them in thermos bottles if this were more convenient. In this

event they recorded the exact hour at which the food was consumed. No restrictions were placed on smoking. No unusual physical activity was permitted, but those subjects who customarily engaged in any type of athletics were allowed to continue [illegible]. Water intake was recorded in approximate amounts and excessive intakes were forbidden.

[illegible] Diet

The Mitmeal I diet was formulated to be similar in proximate composition to the diets customarily consumed by the subjects in order to minimize the adaptation period required. Usual calorie and protein intakes were calculated from a dietary history taken in advance from each subject. The diets were given in liquid form to make possible more precise control of intake and ingredients were selected to facilitate their preparation in standard form without the need to refrigerate or freeze large quantities. Although Mitmeal I was designed for good palatability, it was decided to employ an even more palatable formula in the second series of studies and this has been designated as "Mitmeal 3". The formulas for the two diets are given in Table 3. (Mitmeal 2 was a low protein version of Mitmeal 1 and was formulated for use in another project.)

Each of these formulas provided not only recommended quantities of protein, fat and carbohydrate, but also a balanced complement of other nutrients except vitamin C. The latter was supplied by a standard orange juice preparation. The presently available data on the nutrient content of these diets are given in Table 4. Some discrepancies remain to be resolved between values obtained for Mitmeal 1 by the Wisconsin Alumni Research Foundation Laboratory and by [illegible], particularly in regard to the content of vitamin [illegible], niacine, riboflavin, [illegible] acid and calcium. Analyses are being continued. At least all nutrients recognized as essential are provided in adequate quantity.

Subjects were given the option of including in the diet several [illegible] "sour ball" hard candies, but were required to eat the same number each day of the study period. Caloric intakes were adjusted closely enough to requirements so that there was little change in the weight of the subjects during any of the study periods. The weights at the end of each of the 3-day basal period and the beginning of each day during the stress period are given in Table 5.

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Subjects were given the option of including in the diet several vitamin-free "sour-ball" hard candies, but were required to eat the same number each day of the study period. Caloric intakes were adjusted closely enough to requirements so that there was little change in the weight of the subjects during any of the study periods. The weights at the end of each of the 3-day basal period and the beginning of each day during the stress period are given in Table 5.

Table 3.

Composition of 2,500 Calorie Portions of the Diets

Ingredients	Mitmeal I	Mitmeal III
Toasted soy protein	100 gm.	75 gm.
Powdered skim milk	20 gm.	100 gm.
Dextri-Maltose	217 gm.	142 gm.
Corn oil	73 gm.	92 gm.
Salt	1 gm.	1 gm.
Water	866 cc.	650 cc.
Whole egg	200 gm.	-
Cod liver oil	1.2 gm.	-
Vitamin A palmitate	-	20 mg.
Unsweetened grapefruit and orange juice	700 gm.	700 gm.
Homogenized oatmeal	-	400 gm.
Reconstituted Lemon juice	15 gm.	

Table 4.

NUTRIENT COMPOSITION OF DIETS

Proximate Composition - 100 gm.

Diet	Protein gm.	Fat gm.	Carbohydrate gm.	Ash	Calories
Mitmeal 1 (calculated)	3.96	4.57	14.1	---	112.6
Mitmeal 1 (Wisconsin analysis)	4.32	4.83	15.2	---	121.0
Mitmeal 1 (M.I.T. analysis)	4.17	4.17	14.0	0.631	----
Mitmeal 3 (calculated)	4.04	4.63	14.5	---	115.0

Vitamin Content

Diet	A I.U.	Thiamine mg.	Riboflavin mg.	Niacin mg.	Ascorbic acid mg.
Mitmeal 1 (calculated)	23	0.080	0.064	0.198	13.8
Mitmeal 1 (Wisconsin analysis)	67	0.020	0.038	0.188	5.47
Mitmeal 3 (calculated)	27	0.091	0.155	0.183	10.4

Mineral Content/100 gm.

Diet	Na mEq	K mEq	Ca mg.	P mg.	Fe mg.
Mitmeal 1 (calculated)	1.36	4.00	32	62	0.880
Mitmeal 1 (Wisconsin analysis)	1.71	4.33	66.6	67.8	0.921
Mitmeal 1 (M.I.T. analysis)	1.54	5.20	24	--	--
Mitmeal 3 (calculated)	2.02	5.00	74	86	0.640

Table 5.

Body Weights of Subjects During Basal and Stress Periods

	Baseline				Stress						
					Examination Period Days						
Subject	Initial Weight	Per. #1	Per. #2	Per. #3	1	2	3	4	5	6	7
W.K.F.	72.1	72.1	71.7	72.0	73.0	73.7	73.4	73.2	72.9	72.9	73.0
V.R.J.	91.0	89.4	89.1	89.1	91.0	90.6	89.4	89.8	89.4	89.5	
S.D.C.	66.0	65.5	66.0	65.8	66.4	65.5	65.6	65.3	65.4	65.3	
P.O.Q.	78.0	77.1	77.1	77.0	76.1	75.7	75.9	76.1	76.6	76.9	75.6
M.O.M.	-	76.4	76.0	76.6	76.3	76.2	75.5	74.7	74.9	74.8	
L.S.J.	65.0	65.9	66.4	66.9	65.9	65.0	65.0	65.0	-	65.7	
M.W.G.	81.4	80.2	80.1	80.7	80.9	80.9	80.7	80.3	80.3	80.5	
K.M.D.	69.5	69.1	69.3	69.4	69.3	69.1	68.9	68.95	68.95	68.8	
K.G.J.	..	67.4	68.25	68.0	69.4	68.25	67.75	68.0	67.75	67.75	
C.O.K.	59.5	59.5	59.5	59.5	60.0	59.5	60.0	59.5	59.2	59.2	
C.J.J.	67.8	67.1	66.9	66.9	67.4	67.0	66.9	66.9	66.6	66.4	

Analytical Procedures

Fecal samples were homogenized in a Waring Blendor, a subsample was lyophilized and aliquots were taken by weight for the various analyses. After total weight was determined, an aliquot from the 24-hour urine collection was removed from each 24-hour collection made during the 3-day period and pooled. From this an aliquot was taken for biochemical analyses.

Serum total protein was determined by the density gradient method of Lowry and Hunter (J. Biol. Chem. 159:465, 1945). Serum vitamin A was measured by the ultra-micro method of Bessey et al. (J. Biol. Chem. 166:177, 1946) and ascorbic acid by the Lowry et al. micro adaptation (J. Biol. Chem. 160:609, 1945) of the Roe and Kuether method (J. Biol. Chem. 147:399, 1943).

Urinary creatinine was determined using the Folin and Wu procedure (Practical Physiological Chemistry by Hawk, Oser and Summerson, the Blakiston Co., 12th Ed., p. 506) adapted to the Technicon Auto-analyzer (Technicon Instruments Corp., 1960). The method of Reddy et al. (Metabolism 1:511, 1952) modified by Reddy (Metabolism 6:489, 1954) was employed in the determination of 17-hydroxy steroids in urine.

Urinary and fecal nitrogen were determined by the Kjeldahl method using the Technicon Instrument employing automatic, continuous digestion of serial samples and automatic graphing of the titration results (Technicon Instruments Corp., 1961). Results were checked by the A.O.A.C. macro-Kjeldahl procedure. Sodium and potassium were determined in feces and urine using the Technicon Auto-analyzer flame photometer combination with lithium nitrate as the internal standard (Technicon Instruments Corp., 1961). Inorganic phosphorus in urine and feces was determined as inorganic phosphate using the Fiske and Subbarow procedure (J. Biol. Chem. 66:375, 1925) adapted to the Technicon Auto-analyzer. Urinary and fecal calcium were measured by the Moser method (Anal. Chem. 24:1414, 1956) adapted to the Technicon Auto-analyzer by Skeggs and Stevens (Technicon Instruments Corp., 1960).

In the M.I.T. laboratories the diet was analyzed for nitrogen, sodium, potassium, calcium and phosphorus by the methods previously described. Protein was estimated by Kjeldahl nitrogen x 6.25; fat by ether extraction for 22 hours (A.O.A.C. 9th Ed., p. 287, 1960), and carbohydrate calculated by difference. Crude fiber was determined by standard A.O.A.C. methods (A.O.A.C. 9th Ed., p. 288, 1960).

Data on Mitmeal 1 which was furnished by the Wisconsin Alumni Research Foundation Laboratories was obtained by standard A.O.A.C. methods for the nutrients reported.

Results

A. Experiment I.

a. Initial Basal and Stress Periods

1. Nitrogen - Nitrogen intake ranged from 15.1 to 17.6 gm. per 24 hours depending upon the weight of the subject. The use of the formula diet permitted dietary intakes which were constant throughout all of the study periods and which were within the limits of the weight and volume measurements.

Nitrogen absorption averaged 91.5% (S.D. = ± 3.6%). It was thought that most of the apparent variation in absorption was an artefact caused by variations in the exact period represented by the fecal collection rather than physiological differences. Collection of feces posed problems for some of the subjects, particularly when the marker was delayed in coming through or did not provide a sharp endpoint.

The data for nitrogen balance are given in Table 6. They do not take into account the data for the initial 3-day adaptation and training period although using them would not change the conclusions in any way. All of the nitrogen balances obtained during the basal periods were clearly positive except for two individuals with negative balances during the second basal period. In two of the subjects the lack of a fecal sample made it impossible to calculate nitrogen balance for one of the basal periods. During the two stress periods, nine out of ten subjects showed a marked decrease. Eight of the ten boys were in negative balance during one or both of the two 3-day periods.

Analysis of variance of these nitrogen balance data which are summarized in Table 6 revealed that the variation in nitrogen balance among subjects or between either the two basal or two stress periods was not significant. Comparison between the baseline values and those obtained during the stress periods indicated a difference which is statistically significant at a level of $>$0.01.

If the variation between the basal and stress periods was not due to differences in either intake or absorption, it must, of necessity, be the consequence of increased urinary nitrogen excretion. As shown in Table 7, urinary nitrogen excretion was, in fact, observed to be generally greater during the stress periods. Analysis of variance of urinary nitrogen excretion indicated that differences among subjects or between two basal or two stress periods are far short of statistical significance. The increase in nitrogen excretion in the urine during the stress periods is, however, significant.

Serum protein values averaged 7.30 gm. per 100 ml. and showed no consistent tendency to change during any of the basal or stress periods.

Table 6.

Nitrogen Balance Results

Subject	Basal II	Basal III	Stress I	Stress II
CJJ	+0.39	+2.96	-1.35	-0.63
COK	+2.83	+0.83	+0.23	+0.39
KGJ	+7.54	+5.60	+0.40	-1.68
KMD	+2.68	+2.90	+3.26	+2.03
LSJ	+5.96		+2.39	
MWG	+5.38	+1.91	+3.20	-0.41
MOM	+5.96	-0.22	+2.88	-0.23
POQ		+5.30	-3.86	
SDC	+5.01	+6.63	+0.45	-0.39
VRJ	+2.33	-4.74	-6.86	+2.66
WKF	+4.82	+4.40	-0.92	-0.80

Analysis of Variance

	Degrees of freedom	Sum of squares	Mean square
Periods	3	129.8675	43.29**
Basal II vs. III	1	15.0164	15.02 N.S.
Stress I vs. II	1	0.0657	0.06 N.S.
Basal vs. Stress	1	114.7854	114.78**
Subjects	10	88.1781	8.82 N.S.
Error	26	170.6072	6.56
Total	39	388.6528	

N.S. Not significant

** Significant at the 1% level.

Table 7

Nitrogen Excretion in Urine gm./24 hours

Subject	Basal II	Basal III	Stress I	Stress II
CJJ	13.48	10.95	15.00	13.97
COX	11.69	12.68	13.83	13.12
KGJ	8.91	11.20	15.01	17.33
KMD	11.43	11.23	13.40	14.20
LSJ	6.91	8.25	13.34	16.51
MWG	9.88	13.56	12.20	16.06
HQM	11.00	17.17	13.70	16.24
POQ	11.96	6.50	18.09	
SDC	9.05	6.70	13.28	13.50
VRJ	17.67	11.70	9.89	18.09
WKP	9.28	10.41	15.00	14.93

Analysis of Variance

	Degrees of freedom	Sum of squares	Mean square
Periods	3	165.51	55.17**
Basal II vs. III	1	0.04	0.04 N.S.
Stress I vs. II	1	24.78	24.78 N.S.
Basal vs. Stress	1	140.95	140.95*
Subjects	10	53.07	5.31 N.S.
Error	29	177.08	6.11
Total	43	395.66	

N.S. Not significant
* Significant at the 2.5% level.
** Significant at the 1% level.

2. Sodium and Potassium - Sodium excretion levels averaged 60 mEq per day during the adjustment period and 25 and 20, respectively, on the two succeeding basal periods. During the first stress period the average was 84 and during the second it was 32 mEq per day. Judging from these data alone the relatively low sodium content of Mitmeal 1 is promptly reflected in lowered urinary excretion values. Initial urinary excretion of Na was higher, however, in the first and second stress periods than during the first and second basal periods.

Potassium, on the other hand, increased from 74 mEq per day during the adjustment period to 88 and 97 during the basal periods. It was 108 during the first and 130 during the second stress period.

Until the dietary levels of sodium and potassium have been confirmed and additional subjects have been studied further, statistical treatment of these data does not appear warranted.

3. Calcium - Average values for calcium excretion in the urine during the control periods were 0.034 and 0.032 gm./day for an average of 0.033 (S.D. $= \pm 0.014$). For the stress periods it was 0.048 and 0.045 gm./day with an average of 0.044 (S.D.$=$ $\pm$ 0.189). A simple t test comparison indicates that the differences are not statistically significant ($p < 0.05$). There was no significant difference in calcium absorption between the balance ($\bar{m} = 61.3\%$, S.D. $= \pm 0.014$, $N = 22$) and examination periods ($\bar{m} = 57.7\%$, S.D. $= \pm 0.189$, $N = 21$). Since intake was constant, there was thus a tendency for calcium balances to be lower during the examination periods ($p < 0.05$).

4. Phosphorus - Much the same thing can be said for phosphorus as for calcium. Average values for urinary phosphorus were 0.63 and 0.79 gm. per day in the basal periods and 0.86 and 0.89 gm. during the examination periods. When the average for all basal periods ($\bar{m} = 0.710$, S.D.$= \pm 0.244$, $N = 22$) is compared by the t test with that for the examination periods ($\bar{m} = 0.877$, S.D. $= \pm 0.200$, $N = 21$), the difference is not statistically significant. Consistent differences in absorption were not apparent. While phosphorus balance also tended to be lower during the examination periods, the variance was such that this fell short of statistical significance.

5. 17-Hydroxysteroids - There was no apparent correlation between the values for 24-hour 17-hydroxy steroid excretion in the urine. Some difficulty was encountered with the standardization of the method and further treatment of the data will be postponed until the reliability of the analytical method has been confirmed.

6. Creatinine - The results were used only as an added check on the completeness of urine collections.

B. October Control Periods

As early as possible in the school year, baseline studies were repeated on 5 of the subjects studied in the preceding spring. Although the diet was now Mitmeal 3, the same calorie and nitrogen intake was maintained. This time the subjects settled easily and promptly into the routine of the study and the data from the first collection period were as consistent as those from the third. While most of the analyses have not been completed, those for urinary creatinine and nitrogen given in Table 8 are most encouraging.

Urinary creatinine values for a given subject are highly consistent, indicating completeness of urine collection and the values for urinary nitrogen are not only consistent as 3-day averages, but the daily fluctuations not shown in the table were also relatively small. The average value for all periods was 10.98 compared with 11.67 for all 15 "basal" periods in the preceding spring. The average for the same subjects during the examination periods was almost a third higher, 14.27, and the difference is highly significant.

Table 8

Urinary Creatinine and Nitrogen
October Baseline Periods

	Creatinine gm./day			Nitrogen gm./day		
Subject	Basal I	Basal II	Basal III	Basal I	Basal II	Basal III
COK	1.47	1.49	1.48	9.50	9.53	9.89
KMD	1.59	1.58	1.65	12.69	11.55	11.72
MWG	1.73	1.75	1.69	14.12	12.41	12.32
MOM	1.89	2.08	2.01	12.78	13.57	12.13
WKF	1.62	1.68	1.69	11.66	10.85	11.92

Average 10.98 ± 3.3 (N=15)
Average same
5 subjects
all available
periods during
final examinations
in June 14.27 ± 1.2 (N=9)

$t = 5.456$
$p = < 0.001$

Discussion

It is unfortunate , but inevitable, that time is lost at the start of any new project with delays in funding, receipt of equipment, recruitment and training of personnel, standardization of methods and establishing procedures on a routine basis. Nevertheless, it was possible by the end of the first six months of the project to obtain data clearly supporting the initial hypothesis that psychological stress could be shown to exert a significant adverse effect on the balance of nitrogen and probably other nutrients as well. When interpreted in the light of data previously obtained by the principal investigator in children with varying types of infection, there is every reason to believe that many stresses affecting otherwise healthy young men significantly increase nutrient requirements over those determined in subjects free from such influences.

Thus far, the stresses studied have been acute ones in well nourished individuals. Little is known as to the extent to which an individual would adapt to continued stress of the type studied or indeed whether the "basal" periods represent true baselines in view of the tension under which undergraduates at an institute like M.I.T. are continuously subjected. The slightly lower nitrogen excretion values of the same students early in the fall of their sophomore year compared with the spring of their freshman year may be significant in this regard, but more data are needed. One way of checking this will be to study the nitrogen excretion of graduate students who are about to take their preliminary examinations for the doctorate with the values after this examination has been successfully completed and only thesis work remains.

Until recently nitrogen balance studies have been somewhat limited by the time-consuming Kjeldahl procedure for the determination of nitrogen in urine and feces. The experience of the present project indicates that this is largely overcome by use of the Technicon Auto-analyzer which is capable of analyzing 40 urine samples per hour. This makes feasible the analysis of daily urine samples from large groups of subjects. The use of a formula diet makes it possible to keep nitrogen intakes truly constant for many days. Under these circumstances the limiting factor becomes the collection of fecal samples which must combine several days because of the difficulty of separating fecal collection by period even when carmine or charcoal markers are swallowed.

All available evidence suggests that the effect of stress on nitrogen balance, at least, is to cause an increased urinary nitrogen excretion rather than a decreased intestinal absorption. For the studies now underway and those planned for the future, daily urinary nitrogen excretion will become the criterion rather than 3-day nitrogen balance. This should make it possible to

relate the stress effect more closely to the timing and severity of the stimulus and to handle the ensuing data with greater statistical sophistication.

It will still be necessary to continue fecal collections and analysis until it is clearly established whether the intestinal absorption of any of the essential nutrients is influenced by stress. The data obtained thus far fail to indicate an effect on mineral and electrolyte absorption although urinary excretion appears to be increased for some. Considerable further work will be required before any nutrient can be definitely eliminated from consideration as unaffected by stress.

The possible application of these studies to prolonged space flight was not originally anticipated. For such trips it is important to calculate protein and other requirements with great precision since weight of food supplies or of equipment to produce them will be a major consideration in determining the practicability of such flights. Individual variation can be taken into account by balance studies while the astronauts selected are in training, but unless the effects of the many stresses to which they will be subjected in actual flight are taken in consideration, estimates of requirements might be greatly in error.

Plans for Immediate Future

During October and November three consecutive 3-day baseline periods are being completed with 30 subjects. Some of these will participate in the study of the effects of reversal of night and day which is scheduled for the Christmas vacation and most will be followed during the final examination period in February. Some of them will also take part in the studies of the effect of sleeplessness scheduled for the spring vacation period.

Already one of these subjects has developed a severe upper respiratory infection during which daily urine nitrogen excretion increased significantly. Others will undoubtedly develop infections during the current academic year. With the assistance of N.I.H. Grant No. A-6274, it will be possible to study additional subjects at the moment they acquire an infection and also to investigate thoroughly the effect of each of these stresses on serum amino acid levels. Dr. Guillermo Arroyave, Chief of the Division of Physiological Chemistry of INCAP and an expert on clinical studies of amino acid metabolism, is spending the year as Visiting Associate Professor to assist with this phase of the work. Now that all of the equipment has been delivered, personnel trained, methods standardized and procedures have become routine, the pace of the work has reached the level originally planned.

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